High dose bystander effects in spatially fractionated radiation therapy

Asur, R., Butterworth, K. T., Penagaricano, J. A., Prise, K. M., & Griffin, R. J. (2015). High dose bystander effects in spatially fractionated radiation therapy. *Cancer Letters*, *356*(1), 52-57. https://doi.org/10.1016/j.canlet.2013.10.032

Published in:
Cancer Letters

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
Copyright 2013 Elsevier.
This manuscript is distributed under a Creative Commons Attribution-NonCommercial-NoDerivs License (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits distribution and reproduction for non-commercial purposes, provided the author and source are cited.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

Download date:05. May. 2023

High dose bystander effects in spatially fractionated radiation therapy

Rajalakshmi Asur[1], Karl T.Butterworth[2], Kevin M Prise[2], Robert J. Griffin[1]

[1] Department of Radiation Oncology, University of Arkansas for Medical Sciences, Little Rock, AR 72205

[2]Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, Northern Ireland, United Kingdom

Keywords: GRID, IMRT. Bystander effects, spatially fractionated radiation therapy

Abstract

Treatment of bulky tumors by traditional radiotherapy has certain limitations. Spatially fractionated radiation therapy (GRID) and intensity modulated radiotherapy (IMRT) are examples of advanced modulated beam therapies that help in significant reductions in normal tissue damage. GRID refers to the delivery of a single high dose of radiation to a large treatment area that is divided into several smaller fields, while IMRT allows improved dose conformity to the tumor target compared to conventional three-dimensional conformal radiotherapy. In this review, we consider spatially fractionated radiotherapy approaches focusing on GRID and IMRT, and present complementary evidence from different studies which support the role of radiation induced signaling effects in the overall radiobiological rationale for these treatments.

1. Introduction

The success of traditional radiotherapy in the treatment of bulky or deep-seated tumors is limited by poor blood flow, hypoxia in the tumor, poor depth dose distribution and toxicity to the skin and surrounding normal tissue. Normal tissue cannot tolerate the large radiation doses required to treat the increase in tumor volume successfully. Although significant reductions in normal tissue complication have been afforded through the implementation of advanced modulated beam therapies such as intensity modulated radiotherapy (IMRT) in the clinic, emerging evidence suggests additional benefit may be gained by delivering decreased number of higher doses in some tumor types [2, 24]. An additional approach which has the potential to offer further improvement is spatially fractionated radiation therapy (GRID). GRID describes the delivery of a single high dose fraction to a large treatment area which has been divided into several smaller fields with steep dose gradients thus reducing the overall toxicity of the treatment [46]. In this review, we consider spatially fractionated radiotherapy approaches focusing on GRID and IMRT, and present complementary evidence from different studies which support the role of radiation induced signaling effects in the overall radiobiological rationale for these treatments.

2. Classification of radiation induced signaling effects

The efficacy of ionizing radiation in cancer therapy stems from its ability to induce cell death as a consequence of DNA damage due to energy deposition in the cellular environment. Cellular radiobiological responses are mediated largely through direct energy deposition in cellular DNA or indirectly through reactive oxygen species (ROS) and other free radicals formed during the radiolysis of water [25].

The classical paradigm in radiation biology which focused on nuclear DNA as the sole target of radiation induced damage has been challenged over the last 25 years with an increasing amount of evidence demonstrating radiobiological effects in cells which are not directly traversed by the radiation field. These effects, termed radiation induced bystander effects (RIBEs) generally describe a range of radiation induced signaling effects that have been observed under different *in vitro* and *in vivo* exposure conditions.

RIBEs were first identified by Nagasawa and Little [44] who observed chromosome damage in the form of sister chromatid exchanges in more than 30% of a cell population under conditions in which only 1% of cell nuclei had been targeted using α-particles. Since then, RIBEs have been demonstrated using a range of experimental systems with multiple biological endpoints. Despite increasing evidence in a growing number of model systems, the implications of RIBEs for radiotherapy and cancer risk remain to be fully determined. Whilst conventional approaches to study RIBEs have used techniques somewhat removed from clinical exposure scenarios including media transfer [42] and co-culture models [5, 6], characterization of RIBEs occurring in response to advanced clinical exposures such as intensity modulated radiotherapy (IMRT) and GRID will provide additional understanding of their importance in overall radiobiological response.

RIBEs are primarily radiation induced signaling effects that have been shown to be mediated through direct physical cell contact via gap junction intercellular communication (GJIC) [5] or through the secretion of diffusible signaling molecules into the surrounding medium [11, 43, 57]. The underlying mechanisms mediating response have been extensively studied in a number of model systems and shown to include reactive oxygen and nitrogen species (ROS/NOS) including nitric oxide (NO), cytokines such as transforming growth factor-β (TGF-β) and

interleukin-8 (IL-8), which initiate multiple downstream signaling pathways including the mitogen activated protein kinases (MAPKs) and nuclear factor-κB (NF-κB) [47].

Classification of RIBEs is often dependent on the experimental model and exposures conditions which are being investigated. A recent framework for the classification of more general radiation induced signaling effects based on human radiation exposure scenarios was proposed by Blythe and Sykes [8] in which effects were classified into three categories; bystander, abscopal and cohort effects. Within this framework, bystander effects are defined for human exposure scenarios as radiation induced, signal mediated effects in unirradiated cells within an irradiated volume, exposed to a sufficiently low dose that a portion of cells within the exposed volume, survive [8] . These effects are relevant for whole and partial body exposures to very low doses, such as those from background radiation, high altitude flights and ingested radioactive potassium.

Commented [WU1]: RA/RJG please also cite Butterworth et al, 2013: Clnical Onc review.

The second class of effects are abscopal effects, defined as radiation induced effects in unirradiated tissues occurring outside of an irradiated volume. Abscopal effects have been observed for more than 60 years as systemic radiation effects in some patients following radiotherapy. They do not appear to be dose dependent, making them particularly relevant to the partial body exposures typically delivered during conformal radiotherapy. Abscopal effects are rarely recognized in the clinic and so their importance in radiotherapy response remains controversial [28].

The final class of effects are defined as cohort effects. These describe the component of overall radiobiological response in irradiated cells which is not a consequence of direct energy deposition in the target cell but rather due to communication between cells within an irradiated

volume. Cohort effects are relevant for any exposures where the majority of a cell population is exposed to significant dose and whilst this interpretation is relatively uncommon in the literature, there is increasing evidence that intercellular signaling plays a role in overall radiation response.

Commented [WU2]: RA/RG please insert reference to McMahon et al, here.

Although this framework clearly defines different classes of radiation induced signaling effects, which may potentially impact the overall radiobiological responses, it is unlikely that they occur independently in the advanced clinical scenario where patients are exposed to complex spatially and temporally modulated beam profiles with nearby cells receiving vastly different doses. It is consequently difficult to investigate either of these effects in isolation as the effects may stem from the same or similar cellular signaling origin and may be interpreted as different consequences of the same generalized RIBEs.

3. Observations of RIBEs under modulated beam conditions

Spatially fractionated radiotherapy (GRID)

Spatially fractionated radiotherapy (GRID) refers to the delivery of a single high dose fraction of radiation by dividing a large treatment area into several smaller fields, thus reducing the overall toxicity of the treatment [46]. GRID has been successfully used in the treatment of bulky and deep-seat tumors. GRID may be combined with traditional dose/time fractionated radiation therapy or used along with other treatment modalities, including chemotherapy to achieve better control of bulky tumors and it extends the treatment course minimally.

GRID is not a new technique. In the early 1900s, radiation was performed through a perforated screen to successfully deliver higher than normal doses of radiation, safely and without causing complication due to skin toxicity. In the 1950s, GRID was routinely used along with orthovoltage radiation to treat deep seated tumors with minimal skin and subcutaneous

tissue toxicity [36, 40]. However, with the development of megavoltage radiation, better depth dose distribution and reduced skin toxicity could be achieved and thus the utility of GRID became obsolete, until physicians started facing difficulties in treating bulky and advanced tumors. GRID involves delivery of high doses of radiation through a specially made GRID collimator or a multileaf collimator, such that the entire target does not receive a uniform radiation dose. Instead, only the target directly under the open areas receives irradiation. Recently, it has also been demonstrated that GRID could be applied to deep seated and irregular geometries using the advanced capabilities of a tomotherapy system as well [58].

Clinical results of GRID obtained thus far are very encouraging. Mohiuddin et al. [39, 40] constructed a special GRID collimator using 7 centimeters of lead consisting of 250 equidistant holes. Seventy one patients with advanced bulky tumors were treated with 15 Gy radiation delivered by GRID to various sites including lung, head and neck, gastrointestinal, sarcomas, gynecologic, genitourinary and skin. The patients were treated with GRID alone or GRID followed by fractionated radiation therapy or GRID followed by fractionated radiation therapy and surgery. An overall 75.7% response rate was observed at the end of the study and 78% response rate was observed for palliative treatment and 72.5% for mass effect. A complete response of 16% was observed for all treatments. In another study, 27 patients with advanced head and neck cancer were treated with GRID along with radiation therapy (Group 1) or GRID followed by radiation therapy and planned neck dissection (Group 2) [26]. The overall neck control rate was 93% for Group 1 with a disease specific survival of 50% and morbidity was limited to mild soft tissue damage. In Group 2, an 85% pathologic complete response rate was observed, as well as, 92% neck control rate, 85% disease specific survival and surgical morbidity was limited to wound healing complications. Penagaricano et al. [46] used a multileaf collimator

based GRID design in a cerrobend alloy 7 centimeters in thickness. Fourteen patients with advanced head and neck cancers were evaluated. These patients received GRID followed by chemo-radiotherapy. The overall control rate of the GRID treated tumor volume was 93%, overall neck and primary tumor control rate was 86%. The overall survival was 64% and disease specific survival was found to be 79% and 57% patients exhibited disease free survival.
Although the GRID dose distribution is non-uniform, the regression of the tumor mass receiving GRID has exhibited uniform regression clinically [26, 46]. One plausible explanation might be the enhanced reoxygenation of the tumor following GRID, since the tumor was treated with standard chemoradiation following the GRID dose. A recent study using spatially fractionated microbeam therapy indeed observed marked improvements in tumor oxygenation over the first 2 weeks following exposure [22].

Induction of tumor necrosis factor α and ceramide, as well as down regulation of transforming growth factor β1 have been observed following GRID [50, 51]. In addition, GRID therapy leads to increased cytokine production, resulting in broad systemic effects [46]. It is also possible that bystander effects might play a role in killing adjacent non-irradiated or partially irradiated cells. Studies focusing on the type of inter-cellular communication that might exist between the adjacent cells in the open and closed areas of GRID, might provide valuable information on the mechanisms involved in the promising clinical results observed.

Field Code Changed

GRID-induced bystander effects:

We recently evaluated the ability of spatially fractionated radiation (GRID) in murine carcinoma cells following exposure to a single dose of 10 Gy, analogous to the high single doses used in spatial fractionation [3]. The GRID experiments were performed in a Small Animal Conformal Radiation Research System (SACRRS). A programmable robotic arm is used to

obtain precise positioning of the target/beam, for both therapy and imaging. During the GRID irradiation, the cells are placed on the "palm" of the robot and aligned with the X-ray beam. The GRID pattern of irradiation is then created by programming the robot platform to move perpendicular ~~normal~~ to the X-ray beam direction (Figure 1). The cells were then irradiated at 10 Gy using GRID, to create a pattern of nearly 50:50 direct and bystander exposure pattern of 9 circular fields, 12 mm in diameter with a center-to-center distance of 18 mm. Confluent murine mammary carcinoma (SCK) and head and neck sarcoma (SCCVII) cells were irradiated using GRID. The regions that were exposed to 10 Gy irradiation were considered as "directly irradiated" and the adjacent cells which did not receive direct irradiation, but were exposed to indirect radiation (i.e., scattering) which amounted to a valley dose of approximately 1 Gy, were considered as "bystander cells". The cells were harvested at various time points and re-plated to determine clonogenic survival. A significant bystander killing in cells adjacent to irradiated regions was observed compared to the sham treated controls. The decrease in survival of cells in the adjacent regions was found to be more than that expected from exposure to only background 'valley' or scatter doses, suggesting the existence of true cytotoxic bystander effects following GRID irradiation (Figure 2).

Experiments using real-time PCR arrays specific for mouse DNA damage and cellular stress response pathways were used to determine GRID-induced bystander gene expression changes in mouse head and neck carcinoma (SCCVII) cells [3]. The bystander (GRID adjacent) cells exhibited increased expression of genes involved in DNA repair, cell cycle arrest and apoptosis immediately following exposure or 4 h after exposure. In some instances the increase persisted up to 24 h post GRID irradiation. Increased expression of antioxidant, heat shock and chaperone genes immediately following irradiation or 4 h post GRID exposure in the bystander cells. A

Field Code Changed

significant increase in expression of these genes in the GRID irradiated cells was not observed. In contrast, it has been reported that p53 related genes exhibited minimal activation in bystander cells, while the genes involved in NFκB were activated to equal degrees in direct and bystander cells [20, 21] following alpha particle irradiation of fibroblasts. In these studies, a significantly higher level of the genes encoding Glutathione peroxidase, *Gpx1* and superoxide dismutase, *Sod1* was observed in the bystander cells compared to sham treated controls [3]. These antioxidant enzymes have been known to be important in the cellular defense to oxidative stress [19, 38]. It has been hypothesized that proteins able to withstand freezing and thawing, might be responsible for transmitting the bystander signal from irradiated to naïve bystander cells [27, 31, 43, 45, 52]. Reactive oxygen species [29, 31, 33], growth factors and cytokines [7] have been implicated in the maintenance of the bystander signal. Our results suggest that secreted factors that lead to reactive oxygen species are a very likely candidate for the effects observed, since we observed the greatest increase in expression of antioxidant genes immediately following GRID treatment [3].

Field Code Changed

Intensity modulated radiotherapy (IMRT)

RIBEs have been demonstrated using a wide range of experimental approaches at the single, multi-cellular and whole organism level. Increasingly sophisticated clinical approaches have been driven by technological advancements ~~using radiation microbeams~~ to more accurately target the tumor volume. ~~highly focused low energy, micron sized beam to single cells or sub-cellular targets.~~

Whilst early experimental approaches such as media transfer and cell co-culture have provided clear evidence of radiation induced bystander effects, they have largely been conducted

under conditions which do not accurately represent the multi-cellular environment or replicate radiation exposures *in vivo*.

A number of novel approaches have been taken to investigate RIBEs *in vitro* under exposure conditions which more accurately replicate those during clinical exposures. These approaches have focused on characterizing cellular responses following complex spatial and temporal dose distributions which are delivered during typical advanced radiotherapy such as intensity modulated radiotherapy (IMRT). IMRT allows improved dose conformity to the tumor target compared to conventional three-dimensional conformal radiotherapy (3D-CRT). Clinical evidence for IMRT has shown significant improvements in tumor control afforded by dose escalation along with reduced acute and late toxicity in certain tumor types [53]. Although IMRT improves dose distribution to the tumor target, multiple entry fields results in increased volumes of normal tissue exposed to low dose compared to conventional delivery techniques. These dose baths may have important implications for secondary cancer risk, however, the clinical significance of this remain to be fully determined [23, 48].

Since 2005 several groups have attempted to characterize RIBEs under more clinically relevant exposure scenarios within respect to beam energy, delivery time and dose distributions (Table 1). Using a wedge filter to deliver modulated 6 MV photon beam, Suchowerska et al. [54] demonstrated decreased survival in low dose regions with improved survival in high dose regions with both responses being dependent on intercellular communication between the high and low dose regions . This work was complemented by the subsequent study of Claridge Mackonis et al. [34] who investigated cell survival under 3 different beam configurations using a multi-leaf collimator (MLC); a uniform field, a quarter field (25% of cells exposed) and a striped configuration (25% of the cells exposed in 3 parallel strips). The authors showed differential cell

Commented [WU3]: RA/RJG please add references here as specified.

Field Code Changed

Field Code Changed

survival responses in different regions of the beam profiles compared to uniform exposures. These results showed close agreement with that of Suchowerska et al. [54], demonstrating decreased survival in low dose regions and increased survival in high dose regions.

Several studies have further characterized radiobiological responses to modulated fields under different ~~of~~ beam configurations and energies. Butterworth et al., [10] determined cell survival responses to modulated and non-modulated field configurations delivered using an MLC generated step wedge. This study showed no difference in cell survival response to uniform dose distributions delivered using a uniform field or parallel opposed step wedges and no difference in response to modulated exposures using shallow dose gradients. A further study by Butterworth et al. [11] determined cell survival responses occurring in- and out-of-field following modulated beam exposures in which 50 % of the cells were shielded using a multi-leaf collimator (MLC) as shown in Figure 3. In agreement with data from other authors, significantly reduced survival was observed outside of the radiation field compared to the level of response predicted on the basis of scattered dose alone, along with indications of enhanced survival in-field when the cell populations were free to communicate. Additionally, out-of-field responses were shown to be dependent, at least in part on nitric oxide signaling and field size.

A number of reports are now available that further characterize out-of-field responses in different cell lines at different energies, dose rates and using clinically relevant treatment plans [9, 37, 55]. In addition, these effects have also been demonstrated for clinical proton beams delivered using passive scattering and pencil beam scanning techniques [9].

Field Code Changed

Whilst these studies have provided evidence for RIBEs under clinically relevant beam profiles, they are limited to two dimensions, lacking cellular architecture and physiological

context which may be of significant importance pertaining to the tumor microenvironment. RIBEs have been demonstrated at the whole organism level in a number of *in vivo* model systems under different exposure conditions [13]. Historically, the first *in vivo* evidence of RIBEs was provided by the identification of clastogenic factors obtained from the serum of irradiated patients which showed cell damaging activity when transferred onto cultures of unirradiated lymphocytes [18, 32]. Since then, most experimental models used to investigate RIBEs have involved partial body exposures and are therefore classified as abscopal effects, which have been observed clinically for many years and were originally defined as systemic radiation effects following local radiotherapy [41].

Experimentally, abscopal effects have been demonstrated in a number of models. Camphausen et al. [12], showed significant reduction in the growth of tumors implanted to the dorsal midline when the legs of the animals were irradiated in C57BL/6. In the same model, Koturbash et al. [30] showed induction of DNA damage in skin tissue up to 7 mm away from the irradiated site following partial body exposure. Another important model which has been used to demonstrate the dose and spatial dependency of abscopal effects increasing ~~causing~~ tumorigenesis is the Patched-1 *(Ptch1*$^{+/-}$*)* mouse [35].

Although RIBEs have clearly been demonstrated *in vivo*, the systems in which they have been investigated do not accurately replicate typical exposure conditions during radiotherapy, presenting an important opportunity~~. Whilst next generation higher energy microbeams will provide improved subcellular targeting accompanied with advanced imaging,~~ presenting ~~there is a need~~ to determine RIBEs under conditions analogous to clinical protocols. This may be perhaps~~is potentially~~ possible through the application of tumor bearing animals models in combination with advanced small animal radiation research platforms [56] and presents an

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

exciting opportunity to determine the precise implications of RIBEs for radiotherapy and cancer risk following clinically relevant exposures.

RIBEs are not necessarily restricted to exposure to ionizing radiation. Recently, the ability of genotoxic stress producing agents, other than ionizing radiation, to induce bystander effects have been reported. Chemotherapeutic drugs, such as chloroethylnitrosurea [17] , paclitaxel [1], mitomycin C [4, 49] and phleomycin [4] can induce bystander effects through secretion of media soluble factors. In addition, photosensitizers [16], heat [15] and photodynamic stress [14] have all been reported to cause some type of bystander effect. Therefore, the true nature of bystander effects appears to be a cells stress related phenomenon and not necessarily a unique by-product of radiation damage or a specific type of treatment.

Field Code Changed

Perspective

Evidence from a range of studies focusing on understanding the role of RIBEs under clinically relevant exposure conditions from both GRID and IMRT have shown remarkable similarity in radiobiological response. Steep dose gradients associated with IMRT and GRID overall suggest significantly greater than expected decreases in survival out-of-field and unexpected increases in survival in-field. These observations may have important implications for radiotherapy and a more detailed understanding of the mechanisms mediating response may result in improved tumor control whilst reducing normal tissue toxicity. These benefits may be realized only by defined RIBEs in the context of the tumor microenvironment where, amongst other important factors, oxygen tension is likely to have a significant impact. Understanding the contribution of RIBEs following high radiation dose exposure will facilitate the development of optimized dose delivery and potent chemo-radiation approaches.

References:

[1] J. Alexandre, Y. Hu, W. Lu, H. Pelicano, P. Huang, Novel action of paclitaxel against cancer cells: bystander effect mediated by reactive oxygen species, Cancer Res, 67 (2007) 3512-3517.
[2] S. Aneja, R.R. Pratiwadi, J.B. Yu, Hypofractionated radiation therapy for prostate cancer: risks and potential benefits in a fiscally conservative health care system, Oncology (Williston Park), 26 (2012) 512-518.
[3] R.S. Asur, S. Sharma, C.W. Chang, J. Penagaricano, I.M. Kommuru, E.G. Moros, P.M. Corry, R.J. Griffin, Spatially fractionated radiation induces cytotoxicity and changes in gene expression in bystander and radiation adjacent murine carcinoma cells, Radiation research, 177 (2012) 751-765.
[4] R.S. Asur, R.A. Thomas, J.D. Tucker, Chemical induction of the bystander effect in normal human lymphoblastoid cells, Mutation research, 676 (2009) 11-16.
[5] E.I. Azzam, S.M. de Toledo, J.B. Little, Direct evidence for the participation of gap junction-mediated intercellular communication in the transmission of damage signals from alpha -particle irradiated to nonirradiated cells, Proceedings of the National Academy of Sciences of the United States of America, 98 (2001) 473-478.
[6] E.I. Azzam, S.M. de Toledo, J.B. Little, Oxidative metabolism, gap junctions and the ionizing radiation-induced bystander effect, Oncogene, 22 (2003) 7050-7057.
[7] M.H. Barcellos-Hoff, A.L. Brooks, Extracellular signaling through the microenvironment: a hypothesis relating carcinogenesis, bystander effects, and genomic instability, Radiat Res, 156 (2001) 618-627.
[8] B.J. Blyth, P.J. Sykes, Radiation-induced bystander effects: what are they, and how relevant are they to human radiation exposures?, Radiation research, 176 (2011) 139-157.
[9] K.T. Butterworth, C.K. McGarry, B. Clasie, A. Carabe-Fernandez, J. Schuemann, N. Depauw, S. Tang, S.J. McMahon, G. Schettino, J.M. O'Sullivan, H.M. Lu, H. Kooy, H. Paganetti, A.R. Hounsell, K.D. Held, K.M. Prise, Relative biological effectiveness (RBE) and out-of-field cell survival responses to passive scattering and pencil beam scanning proton beam deliveries, Physics in medicine and biology, 57 (2012) 6671-6680.
[10] K.T. Butterworth, C.K. McGarry, J.M. O'Sullivan, A.R. Hounsell, K.M. Prise, A study of the biological effects of modulated 6 MV radiation fields, Physics in medicine and biology, 55 (2010) 1607-1618.
[11] K.T. Butterworth, C.K. McGarry, C. Trainor, J.M. O'Sullivan, A.R. Hounsell, K.M. Prise, Out-of-field cell survival following exposure to intensity-modulated radiation fields, International journal of radiation oncology, biology, physics, 79 (2011) 1516-1522.
[12] K. Camphausen, M.A. Moses, C. Menard, M. Sproull, W.D. Beecken, J. Folkman, M.S. O'Reilly, Radiation abscopal antitumor effect is mediated through p53, Cancer research, 63 (2003) 1990-1993.

[13] Y. Chai, T.K. Hei, Radiation Induced Bystander Effect in vivo, Acta medica Nagasakiensia, 53 (2008) S65-S69.
[14] A. Chakraborty, K.D. Held, K.M. Prise, H.L. Liber, R.W. Redmond, Bystander effects induced by diffusing mediators after photodynamic stress, Radiat Res, 172 (2009) 74-81.
[15] A. Dabrowska, M. Gos, P. Janik, "Bystander effect" induced by photodynamically or heat-injured ovarian carcinoma cells (OVP10) in vitro, Med Sci Monit, 11 (2005) BR316-324.
[16] J. Dahle, E. Angell-Petersen, H.B. Steen, J. Moan, Bystander effects in cell death induced by photodynamic treatment UVA radiation and inhibitors of ATP synthesis, Photochem Photobiol, 73 (2001) 378-387.
[17] A. Demidem, D. Morvan, J.C. Madelmont, Bystander effects are induced by CENU treatment and associated with altered protein secretory activity of treated tumor cells: a relay for chemotherapy?, Int J Cancer, 119 (2006) 992-1004.
[18] I. Emerit, Reactive oxygen species, chromosome mutation, and cancer: possible role of clastogenic factors in carcinogenesis, Free radical biology & medicine, 16 (1994) 99-109.
[19] Z. Gao, E.H. Sarsour, A.L. Kalen, L. Li, M.G. Kumar, P.C. Goswami, Late ROS accumulation and radiosensitivity in SOD1-overexpressing human glioma cells, Free Radic Biol Med, 45 (2008) 1501-1509.
[20] S.A. Ghandhi, L. Ming, V.N. Ivanov, T.K. Hei, S.A. Amundson, Regulation of early signaling and gene expression in the alpha-particle and bystander response of IMR-90 human fibroblasts, BMC Med Genomics, 3 (2010) 31.
[21] S.A. Ghandhi, B. Yaghoubian, S.A. Amundson, Global gene expression analyses of bystander and alpha particle irradiated normal human lung fibroblasts: synchronous and differential responses, BMC Med Genomics, 1 (2008) 63.
[22] R.J. Griffin, N.A. Koonce, R.P. Dings, E. Siegel, E.G. Moros, E. Brauer-Krisch, P.M. Corry, Microbeam radiation therapy alters vascular architecture and tumor oxygenation and is enhanced by a galectin-1 targeted anti-angiogenic peptide, Radiation research, 177 (2012) 804-812.
[23] E.J. Hall, C.S. Wuu, Radiation-induced second cancers: the impact of 3D-CRT and IMRT, International journal of radiation oncology, biology, physics, 56 (2003) 83-88.
[24] M. Hingorani, W.P. Colley, S. Dixit, A.M. Beavis, Hypofractionated radiotherapy for glioblastoma: strategy for poor-risk patients or hope for the future?, The British journal of radiology, 85 (2012) e770-781.
[25] H. Hricak, D.J. Brenner, S.J. Adelstein, D.P. Frush, E.J. Hall, R.W. Howell, C.H. McCollough, F.A. Mettler, M.S. Pearce, O.H. Suleiman, J.H. Thrall, L.K. Wagner, Managing radiation use in medical imaging: a multifaceted challenge, Radiology, 258 (2011) 889-905.
[26] J.L. Huhn, W.F. Regine, J.P. Valentino, A.S. Meigooni, M. Kudrimoti, M. Mohiuddin, Spatially fractionated GRID radiation treatment of advanced neck disease associated with head and neck cancer, Technol Cancer Res Treat, 5 (2006) 607-612.
[27] R. Iyer, B.E. Lehnert, R. Svensson, Factors underlying the cell growth-related bystander responses to alpha particles, Cancer Res, 60 (2000) 1290-1298.
[28] J.M. Kaminski, E. Shinohara, J.B. Summers, K.J. Niermann, A. Morimoto, J. Brousal, The controversial abscopal effect, Cancer treatment reviews, 31 (2005) 159-172.
[29] M. Konopacka, J. Rzeszowska-Wolny, The bystander effect-induced formation of micronucleated cells is inhibited by antioxidants, but the parallel induction of apoptosis and loss of viability are not affected, Mutat Res, 593 (2006) 32-38.

[30] I. Koturbash, R.E. Rugo, C.A. Hendricks, J. Loree, B. Thibault, K. Kutanzi, I. Pogribny, J.C. Yanch, B.P. Engelward, O. Kovalchuk, Irradiation induces DNA damage and modulates epigenetic effectors in distant bystander tissue in vivo, Oncogene, 25 (2006) 4267-4275.
[31] B.E. Lehnert, E.H. Goodwin, A new mechanism for DNA alterations induced by alpha particles such as those emitted by radon and radon progeny, Environ Health Perspect, 105 Suppl 5 (1997) 1095-1101.
[32] C. Lindholm, A. Acheva, S. Salomaa, Clastogenic plasma factors: a short overview, Radiation and environmental biophysics, 49 (2010) 133-138.
[33] F.M. Lyng, P. Maguire, B. McClean, C. Seymour, C. Mothersill, The involvement of calcium and MAP kinase signaling pathways in the production of radiation-induced bystander effects, Radiat Res, 165 (2006) 400-409.
[34] E.C. Mackonis, N. Suchowerska, M. Zhang, M. Ebert, D.R. McKenzie, M. Jackson, Cellular response to modulated radiation fields, Physics in medicine and biology, 52 (2007) 5469-5482.
[35] M. Mancuso, E. Pasquali, S. Leonardi, M. Tanori, S. Rebessi, V. Di Majo, S. Pazzaglia, M.P. Toni, M. Pimpinella, V. Covelli, A. Saran, Oncogenic bystander radiation effects in Patched heterozygous mouse cerebellum, Proceedings of the National Academy of Sciences of the United States of America, 105 (2008) 12445-12450.
[36] H. Marks, Clinical experience with irradiation through a grid, Radiology, 58 (1952) 338-342.
[37] C.K. McGarry, K.T. Butterworth, C. Trainor, S.J. McMahon, J.M. O'Sullivan, K.M. Prise, A.R. Hounsell, In-vitro investigation of out-of-field cell survival following the delivery of conformal, intensity-modulated radiation therapy (IMRT) and volumetric modulated arc therapy (VMAT) plans, Physics in medicine and biology, 57 (2012) 6635-6645.
[38] J.B. Mitchell, A. Russo, The role of glutathione in radiation and drug induced cytotoxicity, Br J Cancer Suppl, 8 (1987) 96-104.
[39] M. Mohiuddin, D.L. Curtis, W.T. Grizos, L. Komarnicky, Palliative treatment of advanced cancer using multiple nonconfluent pencil beam radiation. A pilot study, Cancer, 66 (1990) 114-118.
[40] M. Mohiuddin, M. Fujita, W.F. Regine, A.S. Megooni, G.S. Ibbott, M.M. Ahmed, High-dose spatially-fractionated radiation (GRID): a new paradigm in the management of advanced cancers, Int J Radiat Oncol Biol Phys, 45 (1999) 721-727.
[41] R.H. Mole, Whole body irradiation; radiobiology or medicine?, The British journal of radiology, 26 (1953) 234-241.
[42] C. Mothersill, C. Seymour, Radiation-induced bystander effects: past history and future directions, Radiation research, 155 (2001) 759-767.
[43] C. Mothersill, C.B. Seymour, Cell-cell contact during gamma irradiation is not required to induce a bystander effect in normal human keratinocytes: evidence for release during irradiation of a signal controlling survival into the medium, Radiation research, 149 (1998) 256-262.
[44] H. Nagasawa, J.B. Little, Induction of sister chromatid exchanges by extremely low doses of alpha-particles, Cancer research, 52 (1992) 6394-6396.
[45] P.K. Narayanan, E.H. Goodwin, B.E. Lehnert, Alpha particles initiate biological production of superoxide anions and hydrogen peroxide in human cells, Cancer Res, 57 (1997) 3963-3971.
[46] J.A. Penagaricano, E.G. Moros, V. Ratanatharathorn, Y. Yan, P. Corry, Evaluation of spatially fractionated radiotherapy (GRID) and definitive chemoradiotherapy with curative intent

for locally advanced squamous cell carcinoma of the head and neck: initial response rates and toxicity, International journal of radiation oncology, biology, physics, 76 (2010) 1369-1375.
[47] K.M. Prise, J.M. O'Sullivan, Radiation-induced bystander signalling in cancer therapy, Nature reviews. Cancer, 9 (2009) 351-360.
[48] J.D. Ruben, S. Davis, C. Evans, P. Jones, F. Gagliardi, M. Haynes, A. Hunter, The effect of intensity-modulated radiotherapy on radiation-induced second malignancies, International journal of radiation oncology, biology, physics, 70 (2008) 1530-1536.
[49] R.E. Rugo, K.H. Almeida, C.A. Hendricks, V.S. Jonnalagadda, B.P. Engelward, A single acute exposure to a chemotherapeutic agent induces hyper-recombination in distantly descendant cells and in their neighbors, Oncogene, 24 (2005) 5016-5025.
[50] S. Sathishkumar, B. Boyanovsky, A.A. Karakashian, K. Rozenova, N.V. Giltiay, M. Kudrimoti, M. Mohiuddin, M.M. Ahmed, M. Nikolova-Karakashian, Elevated sphingomyelinase activity and ceramide concentration in serum of patients undergoing high dose spatially fractionated radiation treatment: implications for endothelial apoptosis, Cancer Biol Ther, 4 (2005) 979-986.
[51] S. Sathishkumar, S. Dey, A.S. Meigooni, W.F. Regine, M.S. Kudrimoti, M.M. Ahmed, M. Mohiuddin, The impact of TNF-alpha induction on therapeutic efficacy following high dose spatially fractionated (GRID) radiation, Technol Cancer Res Treat, 1 (2002) 141-147.
[52] C. Shao, K.M. Prise, M. Folkard, Signaling factors for irradiated glioma cells induced bystander responses in fibroblasts, Mutat Res, 638 (2008) 139-145.
[53] J. Staffurth, A review of the clinical evidence for intensity-modulated radiotherapy, Clin Oncol (R Coll Radiol), 22 (2010) 643-657.
[54] N. Suchowerska, M.A. Ebert, M. Zhang, M. Jackson, In vitro response of tumour cells to non-uniform irradiation, Physics in medicine and biology, 50 (2005) 3041-3051.
[55] C. Trainor, K.T. Butterworth, C.K. McGarry, S.J. McMahon, J.M. O'Sullivan, A.R. Hounsell, K.M. Prise, DNA damage responses following exposure to modulated radiation fields, PloS one, 7 (2012) e43326.
[56] F. Verhaegen, P. Granton, E. Tryggestad, Small animal radiotherapy research platforms, Physics in medicine and biology, 56 (2011) R55-83.
[57] H. Yang, V. Anzenberg, K.D. Held, The time dependence of bystander responses induced by iron-ion radiation in normal human skin fibroblasts, Radiation research, 168 (2007) 292-298.
[58] X. Zhang, Penagaricano, J., Yan, Y., Sharma, S., Griffin, R.J., Hardee, M., Han, E.Y., Ratanatharathom, V. , Application of Spatially Fractionated Radiation (GRID) to Helical Tomotherapy using a Novel TOMOGRID, TCRT Express, 1 (2013).

Figure Legends

Figure 1: A Small Animal Conformal Radiation Research System (SACCRS) was used in the GRID irradiation experiments **[3]**.

Figure 2: Cells were irradiated using spatially fractionated radiation to evaluate bystander effects**.** The cells were irradiated at a peak dose of 10 Gy using a brass collimator to create a GRID pattern of 9 open circular areas, 12 mm in diameter with a center-center distance of 18 mm. The bystander cells were harvested from the valley dose region along the diagonal lines illustrated, which represents about 10% of the total radiation **[3]**.

Figure 3: Experimental setup (a) and schematic representation of dose profile and culture flask configuration (b) for intensity modulated beam experiments. Cells were irradiated in a single T75 culture flask (Intercellular communication intact) or as two T25 culture flasks (Intercellular communication inhbited) using a multileaf collimator (MLC) in which 50% of the cell population were placed out-of-field. Penumbra regions for each of the flask configurations were excluded from analysis **[11]** .